

Immunotherapy of acute myeloid leukemia based on $\gamma\delta$ T cells

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A current major challenge of acute myeloid leukemia research is to develop immunotherapeutic strategies that would be employable in all patients. We recently reported that appropriately stimulated $\gamma\delta$ T cells are fully capable of mediating cytotoxicity against leukemic blasts.

The immune response involves sentinels, such as circulating $\gamma\delta$ T cells (V γ 9V δ 2 T cells), which are capable of recognizing and destroying abnormal cells. V γ 9V δ 2 T cells are T lymphocytes that operate at the interface between innate and adaptive immunity, exhibiting potent MHC-unrestricted cytotoxicity, high potential for cytokine release and a broad-spectrum recognition of cancer cells. Thus, V γ 9V δ 2 T cells are attractive effectors for cancer immunotherapy. V γ 9V δ 2 T cells have the natural ability to distinguish normal cells from “modified” cancer cells both in a TCR-dependent manner through recognition of natural phosphoantigens (PAGs) such as isopentenyl pyrophosphate (IPP, an intermediate of the mevalonate pathway, which is frequently dysregulated in cancer cells), and in a TCR-independent fashion, thanks to the expression of natural killer receptors (NKR) upon the recognition of target cells.

Acute myeloid leukemia (AML) is a heterogeneous disease with variable clinical outcomes. Induction chemotherapy given at diagnosis for the majority of patients has undergone little change in over 30 years. Allogeneic stem cell transplantation offers an example of a setting in which various immune effectors can contribute to the eradication of residual leukemic cells¹ but its use is restricted to a minority of patients. Thus, the development of immunotherapeutic strategies

that would be applicable to all patients appears highly desirable. We have recently reported that human V γ 9V δ 2 T cells specifically recognize and kill AML blasts.²

Phenotypic analyses of V γ 9V δ 2 T cells from AML patients at diagnosis showed that these cells exhibited a bias in their differentiation toward an effector memory phenotype, compared with V γ 9V δ 2 T cells from healthy volunteers (HV), in which the majority of circulating V γ 9V δ 2 T cells have a central memory phenotype.² Effector memory cells are known to display higher functional capacities and a reduced (but existing) proliferation potential. Accordingly, we showed that V γ 9V δ 2 T cells from AML patients had a reduced potential of expansion compared with their counterparts from HVs. Interestingly, AML blasts appeared to be involved in the skewing toward this effector memory phenotype. V γ 9V δ 2 T cells do not seem the only cell compartment to suffer the influence of leukemic blasts. Indeed, a skewed effector profile was also observed in $\alpha\beta$ CD3 $^+$ CD8 $^+$ T cells from newly diagnosed AML patients.³ However, in the latter case, the effector functions of $\alpha\beta$ CD3 $^+$ CD8 $^+$ T cells were deficient due to impaired immunological synapses. When studying the cytotoxic potential of V γ 9V δ 2 T cells from AML patients, we found that these cells responded relatively well to AML blasts (compared with HV

V γ 9V δ 2 T cells). This interaction was monitored both by trogocytosis (namely, the uptake of target-cell material following cell-cell contact) and by direct cytotoxicity. Nevertheless, we cannot exclude that the tumor might have acquired strategies to escape from, or impede, V γ 9V δ 2 T cells effector functions. For instance, the microenvironment generated by AML cells is known to prevent T-cell activation and proliferation⁴ and the direct contact between the chronic lymphocytic leukemia (CLL) cells and T cells has been reported to induce differential gene expression and impair the formation of the immune synapse.⁵ It is therefore conceivable that V γ 9V δ 2 T cells from AML patients may indeed encounter a certain inhibition from blast cells.

When we evaluated the expression of NKR ligands in AML patients at diagnosis to understand why immunity fails, we observed that except for a low expression of ULBP1, none of the NKG2D ligands were present on AML blasts. Conversely, DNAM-1 ligands, PVR and nectin 2, were expressed at the surface of leukemic cells.² Of note, we found that DNAM-1 is partly involved in the recognition and killing of AML blasts, in line with a recent report on hepatocellular carcinoma⁶ and that the cytolytic activity of V γ 9V δ 2 T cells directly correlates with the surface expression of DNAM-1 ligands. In addition, we showed that stimulation with

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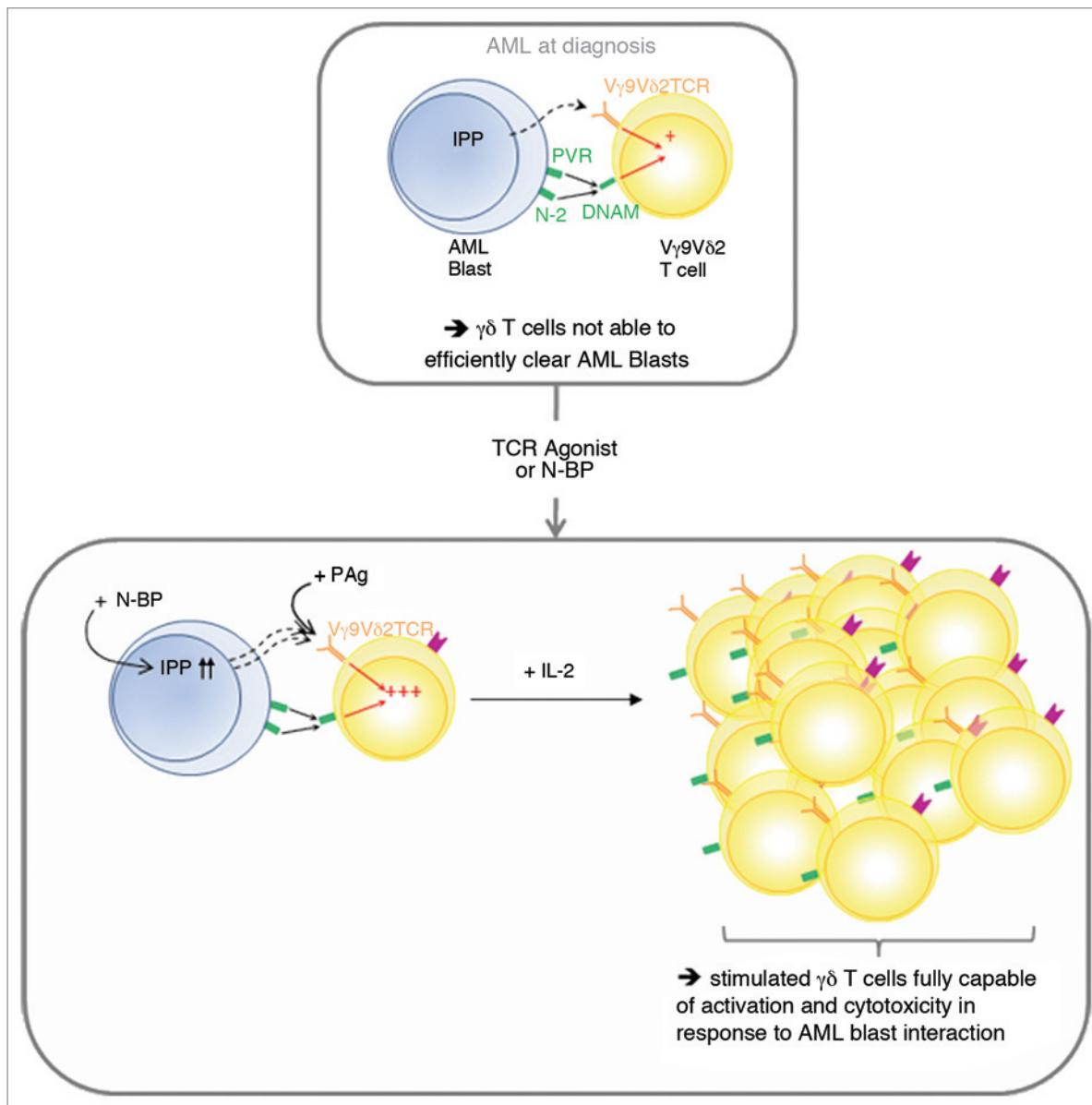


Figure 1. Appropriate stimulation allows an efficient V γ 9V δ 2 T cells anti-leukemic response. At diagnosis and without stimulation, V γ 9V δ 2 T cells recognize acute myeloid leukemia (AML) blasts in a TCR-dependent manner and in a TCR-independent manner, by binding to PVR and nectin 2 via DNAM-1. This induces the differentiation of V γ 9V δ 2 T cells toward an effector memory phenotype, but this is not sufficient to control disease progression. V γ 9V δ 2 T cells can be fully activated in vitro directly, by TCR agonists (synthetic phosphoantigens, PAg), or indirectly, by using amino-bisphosphonates, which lead to the accumulation of the natural PAg isopentenyl pyrophosphate (IPP). Activated V γ 9V δ 2 T cells can be subsequently expanded by an exogenous supply of interleukin-2 (IL-2) for subsequent adoptive transfer into cancer patients.

a synthetic PAg (BrHPP) drastically increases such the recognition of AML blast by V γ 9V δ 2 T cells, as previously described in other cancer settings such as follicular lymphoma.⁷

These results suggest that V γ 9V δ 2 T cells may require priming or co-activation for eliciting optimal antitumor responses. Moreover, these data further strengthen the rationale for the use of V γ 9V δ 2 T cells as therapeutic tools. Hence, the adoptive

transfer of ex vivo expanded V γ 9V δ 2 T cells, or the in vivo stimulation with PAg or another V γ 9V δ 2 T cell agonist such as the aminobisphosphonate zoledronate, may turn out to improve classical therapies for AML. Supporting this hypothesis, two recent studies showed that zoledronate treatment improves in vitro V γ 9V δ 2 T cells functions in chronic myeloid leukemia.^{8,9} Moreover in the setting of allotransplantation for refractory acute

lymphoblastic leukemia (ALL), $\gamma\delta$ T cells were shown to exert a graft-vs.-leukemia effect without the appearance of graft-vs.-host disease (GvHD).¹⁰

Altogether, our work showed that appropriately stimulated $\gamma\delta$ T cells are fully capable of recognizing and killing AML blasts (Fig. 1). Killing of leukemic cells is achieved by means of TCR- and DNAM-1-dependent activation. This study points out the requirements for the

use of V γ 9V δ 2 T cells in therapies against AML. Furthermore, such a targeted cell-based therapy would present the major advantage to be available for most AML patients, and to be potentially devoid of adverse effects such as GvHD.

References

1. Butturini A, Bortin MM, Gale RP. Graft-versus-leukemia following bone marrow transplantation. *Bone Marrow Transplant* 1987; 2:233-42; PMID:3332173.
2. Gertner-Dardenne J, Castellano R, Mamessier E, Garbit S, Kochbati E, Etienne A, et al. Human V γ 9V δ 2 T cells specifically recognize and kill acute myeloid leukemic blasts. *J Immunol* 2012; 188:4701-8; PMID:22467661; <http://dx.doi.org/10.4049/jimmunol.1103710>.
3. Le Dieu R, Taussig DC, Ramsay AG, Mitter R, Mirakid Moud F, Fatah R, et al. Peripheral blood T cells in acute myeloid leukemia (AML) patients at diagnosis have abnormal phenotype and genotype and form defective immune synapses with AML blasts. *Blood* 2009; 114:3909-16; PMID:19710498; <http://dx.doi.org/10.1182/blood-2009-02-206946>.
4. Buggins AG, Milojkovic D, Arno MJ, Lea NC, Mufti GJ, Thomas NS, et al. Microenvironment produced by acute myeloid leukemia cells prevents T cell activation and proliferation by inhibition of NF-kappaB, c-Myc, and pRb pathways. *J Immunol* 2001; 167:6021-30; PMID:11698483.
5. Ramsay AG, Johnson AJ, Lee AM, Gorgün G, Le Dieu R, Blum W, et al. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. *J Clin Invest* 2008; 118:2427-37; PMID:18551193.
6. Toutirais O, Cabillic F, Le Friec G, Salot S, Loyer P, Le Gallo M, et al. DNAX accessory molecule-1 (CD226) promotes human hepatocellular carcinoma cell lysis by V γ 9V δ 2 T cells. *Eur J Immunol* 2009; 39:1361-8; PMID:19404979; <http://dx.doi.org/10.1002/eji.200838409>.
7. Gertner-Dardenne J, Bonnafous C, Bezombes C, Capietto AH, Scaglione V, Ingoure S, et al. Bromohydrin pyrophosphate enhances antibody-dependent cell-mediated cytotoxicity induced by therapeutic antibodies. *Blood* 2009; 113:4875-84; PMID:19278954; <http://dx.doi.org/10.1182/blood-2008-08-172296>.
8. D'Asaro M, La Mendola C, Di Liberto D, Orlando V, Todaro M, Spina M, et al. V gamma 9V delta 2 T lymphocytes efficiently recognize and kill zoledronate-sensitized, imatinib-sensitive, and imatinib-resistant chronic myelogenous leukemia cells. *J Immunol* 2010; 184:3260-8; PMID:20154204; <http://dx.doi.org/10.4049/jimmunol.0903454>.
9. Siegers GM, Felizardo TC, Mathieson AM, Kosaka Y, Wang XH, Medin JA, et al. Anti-leukemia activity of in vitro-expanded human gamma delta T cells in a xenogeneic Ph+ leukemia model. *PLoS One* 2011; 6:e16700; PMID:21304898; <http://dx.doi.org/10.1371/journal.pone.0016700>.
10. Lamb LS Jr, Musk P, Ye Z, van Rhee F, Geier SS, Tong JJ, et al. Human gammadelta(+) T lymphocytes have in vitro graft vs leukemia activity in the absence of an allogeneic response. *Bone Marrow Transplant* 2001; 27:601-6; PMID:11319589; <http://dx.doi.org/10.1038/sj.bmt.1702830>.